2218-Plat
Controlling Protein Binding Specificity by a Conformational Shift
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It is emerging that not only protein structure, but also protein dynamics and conformational equilibria in proteins control to an important extent protein function. This holds for enzymes, where often conformational transitions determine the overall rate, but also for protein-ligand or protein-protein recognition. When nature makes use of the conformational equilibria to implement certain functionality in proteins, the question naturally arises whether redesigning those conformational equilibria would also enable us to manipulate protein properties. In this work, we demonstrate this for protein-protein binding using ubiquitin as a test case. Ubiquitin is a protein with promiscuous binding activity, which is controlled by ubiquitin's global conformational dynamics. These global conformational dynamics are dominated by a collective motion between an open and a closed state. In most complexes, ubiquitin binds preferentially in either the open or the closed state. In native unbound ubiquitin the ratio between the open and closed state is approximately one, suggesting that shifting the equilibrium to either the open or the closed state, would reduce binding to the non-compatible binding partners. Using a molecular dynamics based protein design protocol, we screened 126 core mutants of ubiquitin and identified several that shift the conformational equilibrium between the open and closed state. The change in binding free energy of those mutants to several complexes was verified, both computationally and experimentally (using NMR titration). The observed affinity patterns quantitatively agree with the predictions, thereby showing that, indeed, a shift in the conformational equilibrium enables us to shift ubiquitin's binding specificity and hence its function. Thus, exploiting the fact that conformational selection depends on the concentration of binding-competent states, a novel route towards designing specific binding by conformational shift was demonstrated.

2219-Plat
Reprogramming an ATP-Driven Biological Machine into a Light-Gated Protein Nanocage
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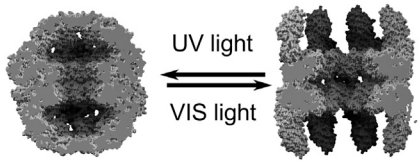
Supramolecular protein assemblies are attractive materials for engineering nanoscale machines with controllable functions. Protein monomers have been engineered to self-assemble into symmetrical complexes, metal-templated structures and cage-like architectures. The mechanistic complexity of protein machines, however, has made it difficult to engineer assemblies with not only new architectures but also desired functions. Here we describe an approach to controlling functional states of a protein assembly with light. The approach uses covalently attached molecular spacers that reversibly switch interatomic distances upon illumination. We applied this strategy to convert an ATP-dependent homo-oligomeric group II chaperonin to a light-driven machine that undergoes large-scale conformational changes between open and closed states visualized by single particle cryo-electron microscopy. The resulting light-gated nanocontainer can capture and release non-native cargos. The design principle of alternately stabilizing conformational states by switching atomic distances illuminates the cooperativity of evolved protein assemblies and provides a strategy for engineering other light-controlled biologically inspired machines.



Platform: DNA Structure and Dynamics

2220-Plat
Sorting Out the Structure of Single-Stranded DNA
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The mechanical properties of single stranded nucleic acids play a vital role in such dynamic processes as DNA replication and RNA folding. While experiments have demonstrated that duplex DNA molecules behave as ideal worm-like chains with a persistence length that can be measured accurately, the unfolded single-stranded form of DNA (ssDNA) has been surprisingly recalcitrant: different biophysical techniques lead to different conclusions. Making matters worse, all-atom molecular dynamics simulations of ssDNA do not agree with experiment. This has motivated new experimental and theoretical work. In recent force spectroscopy and computational studies, McIntosh, Stevens and Saleh propose models where excluded volume influences long-ranged structure, while specific ion interactions induce "wrinkles" at short length scales ("Single-stranded DNA is not a wormlike-chain", Biophys. J. 104, 28). However, direct structural evidence for these models is lacking. Thus, we perform detailed investigations of ssDNA homopolymers using x-ray scattering, single molecule FRET, and quantitative characterization of the ion atmosphere in mono- and di-valent salt. We develop a novel data-driven ensemble optimization technique that allows us to visualize the conformational adaptation of the DNA backbone to changes in the ion atmosphere, including the distinct effects of base stacking and ion valence. Correlation functions obtained from the structure ensembles, without reference to polymer or electrostatic theories, nonetheless show signatures of electrostatic excluded volume and strong ion effects at short distances. We discuss the implications for new polyelectrolyte theories of ssDNA and the biological role of electrostatics in these systems.

2221-Plat
Single Molecule FRET Studies of DNA Hairpin Folding
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Hairpins are the simplest structures for investigating fundamental aspects of nucleic acid folding mechanisms. For hairpins exhibiting two-state behavior, all of the mechanistic information is contained in the transition path, the rare event in single molecule trajectories when the free energy barrier between folded and unfolded states is actually crossed. The first step toward observing transition paths is to determine the transition path time. Average transition path times have recently been directly determined in proteins using the Gopich/Szabo maximum likelihood method (Gopich, Szabo *J. Phys. Chem. B* 2009) to analyze folding/unfolding photon trajectories in single molecule FRET experiments (Chung *et al. Science* 2012; Chung and Eaton *Nature* 2013), while the only experimental study of transition paths in nucleic acids used optical tweezer measurements to determine an upper bound of 50 μs in trajectories for a variety of structures (Neupane *et al*. *Phys. Rev. Lett*. 2012). Neupane *et al.* also reconstructed the free energy surface for an indirect determination of average transition path times from Szabo's analytical theory for diffusive barrier crossing. We use single molecule FRET to study a fast-folding DNA hairpin with 2 A-T and 2 G-C base pairs in the stem and 21 T's in the loop immobilized on a polyethylene glycol-coated glass surface via a biotin-streptavidin-biotin linkage. The folding time for this hairpin in 500mM NaCl is 530 μs. Maximum likelihood analysis of 780 transitions in photon trajectories with an average detection rate of 750 photons/ms yields an upper bound of 4 μs for the average transition path time, compared to the value of ~3.6 ± 0.8 μs predicted by Neupane *et al*. from hairpins with 9-30 base pair stems and 4 T's in the loop. Current experiments use viscogens to slow the transition path for directly determining the actual value.

2222-Plat
Friction and Interactions between Bare DNA Molecules: The Role of DNA Handedness
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Cellular DNA is packaged through a range of re-modeling proteins. As a result, disparate regions of the genome may find themselves in close contact with one another. Here, we address an important and emerging issue resulting from this fact: what happens when DNA becomes entangled? Is it possible that the mechanical properties of DNA can influence interactions of the genome even in the absence of proteins? Recent experiments have suggested that the handedness of DNA may affect the stability of DNA-DNA pairs.
Using a single-molecule approach, we employ a unique 4-way optical trap to wrap two separate DNA molecules around one another. By displacing optically trapped beads, it is possible to slide one DNA molecule along the other. Using triangulation, we are then able to track the location at which the DNA molecules are entwined. This analysis reveals that a small but clear friction is present only when sliding the DNA molecule through a right-handed wrap. Moreover, when sliding the DNA further, tension is built up and then released, giving rise to an abrupt stick-release force pattern. Strikingly, the latter is once again found predominantly in the case of a right-handed wrap. By staining the DNA molecules with the force-sensitive fluorescent dye Sytox, we are able to both visualize and measure this build-up and release of force. The stick-release behavior